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A DISEASE OF PELARGONIUMS CAUSED BY *XANTHOMONAS PELARGONII*

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Abstract

A study of bacterial blight of pelargoniums in naturally and experimentally infected plants is reported.

The causative organism *Xanthomonas pelargonii*, hitherto not described in Australia, was isolated. The cultural, biochemical and antigenic characteristics of the bacterium are presented.

Introduction

Symptoms of blight were observed in pelargoniums (*Pelargonium hortorum*) in July 1958, and in July and November of the following year. The plants were potted cultivars 'Reverend Atkinson' and 'Lady Ilchester' grown from cuttings originally obtained from the Royal Botanic Gardens, Melbourne, and from a commercial firm in New South Wales. The symptoms of the disease indicated the possibility of an infection with *Xanthomonas pelargonii* (Brown) Starr and Burkholder.

A perusal of literature reveals that diseases of pelargoniums have been described by a number of workers, and Hellmers (1952) presented a short history of those investigations. According to records, as far back as 1884, a disease of pelargoniums in France was suspected to be of bacterial origin. Hellmers (1952) in Denmark was able to prove that two different syndromes, namely stem lesions described by some as 'bacterial stem rot of pelargonium' and leaf spots observed by other workers as 'bacterial leaf spot of pelargonium', can be the expression of an attack by the same pathogen, namely *X. pelargonii*.

According to a recent publication (New South Wales Dept of Agric. 1959) 'Geraniums known to the botanist as species of Pelargonium . . . have found favour once more'. The re-appearance of those plants in gardens focussed attention on diseases affecting them under local conditions. Apparently in recent years, a bacterial disease of pelargoniums has been noticed in Victoria (Harrison, personal communication) and it is believed that the disease has been present in New South Wales since 1955 (Sutton, personal communication).

As no published records of the isolation in Australia of the causative organism were found, a detailed study was made of the disease under local conditions.

Symptoms in Naturally Infected Plants

Early in the disease, large brown to black lesions appeared on the basal part of the main stem, while the higher part looked dry, shrunken and hollow and had a 'corrugated' appearance. The terminal part of the stem and the petioles seemed at first unaffected but the basal leaves withered. At a later stage, as the infection spread upwards to the tip of the stem, the petioles and the stem developed a brown

discolouration, while the leaves blackened and gradually fell off. The whole plant was stunted and finally withered.

The root system, although dry and discoloured, seemed to be the least affected part of the plant.

Careful examination of naturally infected plants failed to reveal the presence of distinctive leaf spots usually associated with pelargonium blight.

Bacteriological

Materials and Methods

For the isolation of the organism plants in the early stage of the disease were chosen. The stem slightly above a black lesion was washed with 10% mercuric chloride, then with 70% alcohol, followed by a few washings with sterile tap water. Isolations from scrapings of the tissue or from the sap were made by plating on brain-heart agar ('Difco' product) or potato-dextrose agar and on wort agar.

After isolation, the cultures were maintained at 4°C on brain-heart agar under paraffin oil and on Dorset egg medium in screw capped Bijou bottles which were firmly closed after abundant growth was obtained. Subcultures were made approximately every 2 months. Biochemical tests were performed as follows:

Carbohydrate fermentations were determined using carbon compounds in a synthetic peptone-free medium (Dowson 1957) observed for 28 days.

Other tests were carried out according to directions given by Mackie and McCartney (1953) unless otherwise stated. The indole test was performed after 7 days, using xylol for extraction. Methyl red and Voges-Proskauer tests were carried out after 4 days incubation at 25°C, and creatine was used in the Voges-Proskauer test. Nitrate reduction was determined in a medium with 0·2% potassium nitrate and the cultures were spot-tested after 1, 2 and 5 days incubation. Hydrogen sulphide production was observed on brain-heart agar using lead acetate paper.

The serological techniques used were those recommended by Kauffmann (1954) except for the method of producing diagnostic antisera, which was according to our own previously described procedure called 'shock treatment' (Mushin, Naylor and Lahovary 1959). Steamed saline suspensions from growth of two isolates on brain-heart agar served as somatic antigens. Prior to injections, the sera of the rabbits were shown to be free of normal agglutinins against the organism under investigation and against the surface antigens α and β (Mushin, Naylor and Lahovary 1959). The somatic antigens were inoculated into the rabbits in 7 intravenous injections each of 0·5 ml within 8 days. The animals were test bled the second day after the final injection and, if the titre was satisfactory, the rabbits were bled out from the carotid artery on the third day. The glycerolated sera were stored at 4°C.

Results

Microscopic examination of hand-cut sections of stem lesions showed the tissue cells to be filled with bacteria but no fungal hyphae were observed.

Cultural Characters: After 48 hours incubation on brain-heart agar the colonies were slightly yellowish, circular and semi-transparent, measuring 1 to 2 mm in diameter. The confluent growth on brain-heart agar was also yellowish, while on potato-dextrose agar the pigment was distinctly yellow and the colonies were mucoid.

Morphology: The organism was a Gram-negative rod, 0·5μ x 1 to 1·5μ in size, showing monotrichous flagellation.

Biochemical Characters: Acid was formed in glucose, sucrose and galactose in 3 to 5 days and in lactose after 10 days, while salicin, maltose, rhamnose, mannitol and glycerol were not fermented. Litmus milk was peptonized in 4 days and the surface of gelatin was liquefied in 5 days.

The indole, methyl red and Voges-Proskauer tests were all negative. Urea was either not hydrolysed or very slightly. The citrate test was positive. Nitrate was not reduced to nitrite. Hydrogen sulphide was produced.

Serology: Glycerolated "O" sera agglutinated heated suspensions of the isolated organism up to a titre of 1280. Slide agglutinations occurred when serum was mixed with saline suspensions of the living bacteria, thus indicating the absence of a mucoid surface antigen which tends to block the reaction.

Table 1

Titres of 'O' Agglutination Tests with Unabsorbed and Absorbed Antisera Against X. pelargonii

Antiserum	Titres against suspension of:		
	X. pelargonii	*X. campestris*	*X. phaseoli*
Unabsorbed	1280	640(3), 40(1)	160(1), —(3)
Absorbed with suspension of:			
X. pelargonii	—		
X. campestris	1280	—	—
X. phaseoli	1280	—	—

— = Titre < 20. Numbers in parenthesis refer to the number of strains giving a particular reaction.

As shown in Table 1, agglutination tests indicated a close serological relationship between *X. pelargonii* and 3 strains of *X. campestris* and a slight affinity with one strain of *X. campestris*. Antigenic relationship was also demonstrated between *X. pelargonii* and one out of 4 *X. phaseoli* strains tested. 5 other Xanthomonas species, namely *X. juglandis, X. vesicatoria, X. carotae, X. incanae* and *X. albilineans*, were not agglutinated by *X. pelargonii* antiserum.

Absorption of the serum by *X. campestris* and *X. phaseoli* antigens did not lower the titre of *X. pelargonii* antiserum against its homologous antigen, showing the presence of an additional major antigenic factor in the structure of *X. pelargonii*.

Plant Pathogenicity Tests

Methods of Propagating Test Plants

Cuttings were taken in the autumn from healthy pelargonium plants (*P. hortorum*) which had shown for several seasons normal growth cycles. The base of each cutting was dusted with a rooting powder containing 5 p.p.m. indole butyric acid and they were inserted into a medium made up of equal parts of standard grade vermiculite and perlite, both products being of local manufacture. The medium was contained in new flats which were treated with copper naphthleneate to inhibit fungal growth and the foliage was sprayed with systemic insecticide.

After development of sufficient root system, the test plants were placed in 3½″ terracotta pots. These contained washed river sand obtained locally from Fisherman's Bend, granulated peat from West Germany and fertilizer. The plants were watered individually with a can and no 'bottom soaking' was employed.

Experimental Infection of Pelargonium Plants

Two series of experiments were set up. Various cultivars of *P. hortorum* were used, namely 'Lady Ilchester', 'Reverend Atkinson', 'Welcome', 'Festival', 'Plum Rosebud' and 'French Bouquet'.

In the first set of experiments 9 plants were placed in moist chambers in a glasshouse regulated at 25°C for 24 hours before and after inoculation. 6 plants were inoculated into the stems, using a needle dipped in a thick saline suspension of the test organism grown on moist brain-heart agar for 48 hours. 3 control plants were pricked with a sterile needle.

After 2 weeks, the inoculated plants showed water marks and 'corrugated' lines on stems (Pl. VI, fig. 1, 2). 2 weeks later, the symptoms were more pronounced, a dry type of rot was evident and the plants were slightly dwarfed beside the controls (Pl. VI, fig. 3). 6 weeks after the inoculation, the stems had rotted and the leaves were withered, but at no time were any distinct leaf lesions observed. The inoculated organism was recovered from the stems in pure culture.

Another series of experimental infection of plants was set up in summer, at the end of December. Here the procedure of inoculation differed slightly in that a small gauze plug, dipped in a bacterial broth culture was inserted into a needle puncture in the stem. This provided a reservoir of the organism and protected from drying (Kivilaan and Scheffer 1958). 9 inoculated plants and 3 controls were covered with gauze and placed in 2 separate groups out-of-doors in a shaded area of the garden.

Records of temperature and humidity readings at 9 a.m., 3 p.m., and 9 p.m. were obtained during a 3-week period of observation.

Table 2

Temperature and Humidity Records during an Out-of-doors Plant Inoculation Test

Time	Temperature °F		% humidity	
	Highest	Lowest	Highest	Lowest
9 a.m.	90	61	84	28
3 p.m.	97	61	94	21
9 p.m.	88	61	77	31

It can be seen that the temperature at times indicated in Table 2 ranged from 61°F to 97°F, while the humidity varied between 21 and 94%.

Within 2 to 3 weeks of inoculation, symptoms of dry brown rot appeared on stems of the infected plants and the leaves gradually withered, but no leaf lesions were evident. The causative organism was recovered in pure culture.

Additional Tests

It was desirable to confirm the identity of the isolated organism, suspected of being *X. pelargonii*, by comparing it with a type culture. Australian quarantine regulations do not permit the importation of cultures of plant pathogenic bacteria not known to be already established in this country. Therefore, arrangements

were made for our cultures to be examined by two overseas authorities. Dr D. W. Dye of Auckland, New Zealand, and Professor A. W. Dimock of Ithaca, U.S.A., both confirmed the identity of the bacterium as *X. pelargonii.*

Additional information was given by Dr Dye who used our isolates to inoculate plants under environmental conditions different from those in our experiments. Young leaves of actively growing plants were inoculated with broth cultures of our isolates. The plants were placed in a temperature-controlled cabinet in which sufficient water mist was supplied to keep leaf surfaces wet without considerable run-off (Dye, personal communication). Under those conditions, leaf spots were obtained in the form of angular to roughly circular water-soaked areas sometimes surrounded by a yellow halo. Later the lesions became brown to black in colour and dry (Pl. VI, fig. 4).

Conclusions

From the description of the bacterium and the blight symptoms reproduced in host plants it was concluded that the organism described is *X. pelargonii.* Therefore the disease is apparently endogenous in Australia.

Discussion

It was shown (Kivilaan and Sheffer 1958) that various factors may affect the development of bacterial stem rot (bacterial blight) of pelargoniums. Under our local conditions the stem rot was observed and was easily reproduced by infecting healthy pelargonium hosts with *X. pelargonii.* Leaf spots were not reported in naturally infected plants but they appeared in New Zealand in plants inoculated with our isolates under favourable conditions of high humidity. The morphology, cultural and biochemical characters of the isolated strain of *X. pelargonii* were similar to those described by other workers. A minor difference was noticed in the failure of the local strain to ferment glycerol as described by Hellmers (1952), while mannitol fermentation is apparently variable (Starr, Volcani and Munnecke 1955).

It was of interest to compare the results of our serological tests with those performed by Elrod and Braun (1947A). Their technique differed in that living bacteria were used as antigens and the mucoid material was extracted from the cells with warm saline. In their grouping of Xanthomonas species based on antigenic structure, *X. pelargonii* was placed in a group of strains related to *X. campestris* and *X. phaseoli.*

In our experiments, using steamed suspensions of freshly isolated strains of *X. pelargonii,* the somatic antigens seemed to be devoid of mucoid material. A close serological relationship was shown with 3 out of 4 strains of *X. campestris* and a slight affinity with one only out of 4 strains of *X. phaseoli.* This discrepancy in serological results may be due to differences in strain specificity or in the application of different techniques. Recently, Sloda and Cleverdon (1959) also indicated a serological affinity between *X. pelargonii* and *X. campestris.* The lack of antigenic relationship between *X. pelargonii* and a few other species tested in this study confirms the results obtained by the above workers.

Elrod and Braun (1947B) pointed out the possible discrepancies when species identification is based on results of pathogenicity tests and serological reactions. Dye (1958) has shown that host specificity, on which the classification of Xanthomonas species is dependent, may not be a stable character. There seems to be a possibility that further studies of host-parasite relationships amongst plant

pathogenic bacteria may widen the host spectrum of various species, thus undermining host-specificity as the determinative factor in classification within the genus. On the other hand, a thorough bacteriological examination of large numbers of bacterial strains with emphasis on antigenic analysis may provide a sounder base for species identification.

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Explanation of Plate

Plate VI

Fig. 1 and 2—Close-up of a stem of pelargonium (*Pelargonium hortorum*) 4 weeks after inoculation.

Fig. 3—Pelargonium plants (*P. hortorum*) : healthy control and 4 weeks after inoculation.

Fig. 4—Leaf spots on pelargonium 6 days after inoculation. Centre top are leaves of *P. peltatum* and lower of *P. hortorum.*